Chromatin reorganisation in Epstein-Barr virus-infected cells and its role in cancer development

Article (Accepted Version)

- Key EBV transcription factors hijack host cell factors to reprogramme B cells.
- This is mostly directed through binding to host gene enhancers and super-enhancers.
- Binding can promote or disrupt enhancer-promoter contacts to turn genes on or off.
- EBV activation of upstream *MYC* enhancers may promote upstream *MYC* translocations.
- Enhancer hub disruption by EBV can initiate long term gene silencing.

Chromatin reorganisation in Epstein Barr virus-infected cells and its role in cancer development

Michelle J. West

School of Life Sciences, University of Sussex, Brighton, BN1 9QG, UK

Correspondence to Prof Michelle J. West

Email:m.j.west@sussex.ac.uk

Tel: +44 (0)1273 678404

Abstract

The oncogenic Epstein-Barr virus (EBV) growth transforms B cells and drives lymphoma and carcinoma development. The virus encodes four key transcription factors (EBNA2, EBNA3A, EBNA3B and EBNA3C) that hijack host cell factors to bind gene control elements and reprogramme infected B cells. These viral factors predominantly target long-range enhancers to alter the expression of host cell genes that control B cell growth and survival and facilitate virus persistence. Enhancer and super-enhancer binding by these EBNAs results in large-scale reorganisation of three-dimensional enhancer-promoter architecture to drive the overexpression of oncogenes, the silencing of tumour suppressors and the modulation of transcription, cell-cycle progression, migration and adhesion.

Introduction

Epstein-Barr virus (EBV) is an oncogenic γ-herpesvirus responsible for 200,000 lymphoma and carcinoma cases worldwide per year. Despite its oncogenicity, EBV infection is usually harmless and >95% of the worldwide population carry EBV as a life-long asymptomatic infection. B cell infection by EBV results in growth transformation and the establishment of a persistent latent infection in memory B cells [1]. An effective cytotoxic T lymphocyte response controls the number of EBV-infected cells and protects from tumour outgrowth [2].

Initial B cell growth transformation by EBV *in vivo* and EBV infection *in vitro* results in the expression of all latent genes including six nuclear proteins (Epstein-Barr virus nuclear antigens; EBNAs, 1, 2, 3A, 3B, 3C and leader protein), three latent membrane proteins (LMP1, 2A and 2B), numerous viral microRNAs and two highly-expressed non-coding RNAs. Five latent proteins (EBNA1, 2, 3A, 3C and LMP1) are critical for the transformation of B cells [3-6]. EBNA2, 3A and 3C play key roles in the B-cell transcriptional reprogramming which is crucial for the outgrowth and survival of EBV-immortalised cells. Although not required for B cell transformation, EBNA3B cooperates in the regulation of large numbers of cellular genes [7]. This review focuses on recent advances in our understanding of how these four viral transcription factors (TFs) deregulate host cell genes through the hijacking of long-range regulatory elements and the reconfiguration of enhancer-promoter architecture to induce growth transforming cancer-promoting events.

EBV TFs associate with host cell factors to reprogramme the B cell transcriptome

EBNA2, EBNA3A, EBNA3B and EBNA3C alter the expression of thousands of B cell genes involved in key cellular processes such as lymphocyte activation, signalling, apoptosis,

transcription, cell cycle, cell adhesion and migration, including many proto-oncogenes and tumour suppressors. These EBV TFs do not bind DNA directly and bind gene control elements through interactions with B cell DNA-binding proteins. All four proteins bind the Notch signalling pathway TF, RBPJ (RBP-Jκ, CBF1, CSL) [8,9]. The association of EBNA2 and EBNA3 proteins with RBPJ is mutually exclusive and EBNA2 and EBNA3 proteins competitively bind to RBPJ-bound chromatin sites in the B cell genome [10-12]. The interaction between EBNA2, EBNA3A or EBNA3C and RBPJ is critical for EBV immortalised cell growth [13-16]. EBNA2 and EBNA3C also bind the ETS family member PU.1 [17,18]. More recently, EBNA3C binding to interferon regulatory factors (IRF) 4 and 8 and EBNA3A, EBNA3B and EBNA3C binding to the Runt-related transcription factor (RUNX) partner, core binding factor β (CBF β) was demonstrated [19,20].

EBNA2 is a transcriptional activator that interacts with histone acetyl transferases (HATs), the SWI/SNF chromatin remodeller and the basal transcription machinery, although it can also repress gene transcription through unknown mechanisms [21]. EBNA3A, EBNA3B and EBNA3C comprise a family of distantly-related genes and can repress and activate transcription individually and co-operatively [7]. EBNA3A and EBNA3C associate with the co-repressors C-terminal binding proteins 1 and 2 (CtBP) [22-24] and EBNA3C also binds the histone deacetylase co-repressor complex components HDACs 1 and 2, Sin3A and NCoR [25,26]. Gene silencing by EBNA3A and EBNA3C involves recruitment of polycomb repressor complexes (PRC1 and 2) to target gene loci and the deposition of the histone H3 trimethyl lysine 27 (H3K27me3) silencing mark by the PRC2 methyltransferase EZH2 [27-29]. EBNA3A, EBNA3B and EBNA3C can also activate cellular genes and EBNA 3C associates with the HAT p300, implicating histone acetylation in its mechanism of gene activation [7,30].

EBV TFs predominately target long-range cellular gene regulatory elements

EBNA2 and EBNA3 family proteins regulate viral transcription via promoter elements [31-34], but most cellular target genes do not possess EBNA-responsive promoters. Chromatin immunoprecipitation-sequencing revealed that in an EBV immortalised lymphoblastoid cell line (LCL), 86% of EBNA2 human genome binding sites were in fact outside of promoter regions (>2 kb from a TSS) [35]. Similarly in a Burkitt's lymphoma (BL) cell line, 81% of EBNA2 and 89% of EBNA3 family binding sites were > 2kb from cell gene TSSs [36]. The majority of these EBNA binding sites coincided with histone modifications that characterise enhancer elements, indicating that EBNA2 and EBNA3 proteins were likely to reprogramme B cell transcription by predominantly targeting enhancers. A number of subsequent studies using tagged versions of EBNA3A, EBNA3B and EBNA3C confirmed these results [12,20,37,38]. Motif enrichment analysis of DNA sequences at EBNA2, 3A, 3B and 3C binding sites also identified potential new DNA targeting partners [12,20,37,38]. Of these, IRF4 and the RUNX partner CBFβ have now been confirmed as interacting proteins that are required for the targeting of specific cellular genes by EBNA3C and EBNA3B and EBNA3C, respectively [12,20].

A number of the enhancer regions bound by EBNA2 and EBNA3 proteins display characteristics of 'super-enhancers' [39,40]. These highly-active regulatory regions represent large clusters of individual enhancer peaks containing binding sites for multiple cell-type specific TFs and high levels of histone H3 acetylation [41]. Super-enhancers play a critical role in driving high-level expression of lineage-specific genes and specific super-enhancers are often activated in cancer cells. This makes super-enhancers an attractive target for drugs that can be used to block their activity as drivers of cancer cell growth, such as the bromodomain

inhibitor JQ1 and inhibitors of the RNA polymerase II kinase CDK7 [42-46]. RNAs transcribed at EBV super-enhancers (eRNAs) have also been shown to be important for enhancer function, in common with eRNAs found at non-viral TF bound enhancers [47,48].

Overlap in EBNA2, EBNA3A, EBNA3B and EBNA3C binding

ChIP-sequencing analysis of EBNA2, EBNA3A, EBNA3B and EBNA3C identified extensive overlap in their binding sites. This is perhaps predictable given their shared cellular TF binding partners e.g. RBPJ. It is also consistent with the co-operative and/or antagonistic nature of gene regulation by these EBNAs [7,29,49]. The extent of binding overlap was reported for a BL cell line as 25% of all binding sites shared by EBNA2 and EBNA3 family proteins, with an estimated 80% of genes closest to EBNA2 binding sites also closest to an EBNA3 protein binding site [36]. Binding analysis of individual tagged EBNA3A, EBNA3B and EBNA3C proteins in LCLs found 21-26% binding overlap between different EBNA3 proteins with 37% of genes closest to EBNA2 binding sites also closest to a binding site for an EBNA3 protein [12]. It is therefore likely that the co-regulation of B cell genes by these EBNAs is mediated via their association with the same regulatory element or to different elements that target the same gene.

EBNA2 and EBNA3 proteins regulate enhancer-promoter interactions

The effects of EBNA2, EBNA3A, EBNA3B and EBNA3C binding to long-range elements on the three-dimensional associations between cell gene enhancers and promoters has begun to be investigated using the chromosome conformation capture (3C) family of techniques.

Initial studies focused on the mechanism of activation of the *MYC* proto-oncogene by EBNA2, a key growth promoting event that occurs shortly after EBV infection [50]. ChIP-sequencing detected EBNA2 binding at multiple super-enhancers and enhancers in a 3 Mb region around *MYC* [35,36]. 3C analysis demonstrated that EBNA2 increased interactions between a -428 kb EBNA2-bound super-enhancer and the *MYC* promoter [35]. Further studies on the effects of EBNA2 on the wider *MYC* enhancer-promoter interaction landscape used 4C (circularised chromosome conformation capture) to capture all *MYC* promoter-interacting fragments in the absence or presence of EBNA2 [51]. 4C detected *MYC* promoter interactions with multiple EBNA2-bound upstream and downstream enhancers and showed that EBNA2 induced widespread changes to the three-dimensional arrangement of the *MYC* locus [51]. In the presence of EBNA2, *MYC* enhancer-promoter interactions were increased over a large upstream region encompassing multiple enhancers and super-enhancers, consistent with previous reports, but surprisingly EBNA2 reduced downstream enhancer-promoter interactions [51] (Figure 1). Upstream enhancer interactions were dependent on the presence of the SWI/SNF ATPase BRG1 which is bound and recruited by EBNA2 [51-53]. SWI/SNF is important in maintaining downstream *MYC* enhancer-promoter interactions in leukaemic cells and is required for the function of multiple distal lineage-specific enhancers [54,55]. Similar directional changes to *MYC* enhancer-promoter architecture were induced on infection of primary B cells by EBV that differed from the downstream interactions induced on short-term B cell activation by CD40 ligand and IL-4 [51]. This indicates that EBV activation of *MYC* involves distinct spatial reorganisation of chromatin. Multiple cell TFs are co-localised with EBNA2 at *MYC* enhancers including RBPJ, but which TFs are crucial for EBNA2 binding to *MYC* enhancers is not known. EBNA3A and EBNA3B also bind to *MYC* enhancers, but their effect on *MYC* enhancer-promoter interactions has not been studied [37,38].

Interestingly, in EBV-positive BL, the *MYC*-immunoglobulin translocations that characterise BL have a breakpoint located in the upstream *MYC* enhancer region activated by EBV [51]. EBV also upregulates the expression of the activation induced cytidine deaminase enzyme (AID), known to be an inducer of *MYC* translocations [56,57]. It has therefore been proposed that the specific activation of upstream *MYC* enhancers by EBV may increase the susceptibility of this region to translocations induced by off-target AID activity and could represent an initiating event in the genesis of BL [51].

Further studies characterised an EBNA2-bound and RBPJ-dependent *RUNX3* super-enhancer 97 kb upstream of the gene TSS [40]. *RUNX3* is a direct target of EBNA2, likely to play an important role in promoting growth, at least in part by its downregulation of Runx1c expression, which is growth inhibitory in B cells [58-60]. Genome-wide promoter-enhancer analysis in an EBV-immortalised cell line detected high levels of interactions between the *RUNX3* super-enhancer region and *RUNX3* promoters, providing evidence for the presence of RUNX3 super-enhancer-promoter interactions in EBV-infected cells [40,61] (Figure 1). The *RUNX3* super-enhancer is also bound by EBNA3B and EBNA3C and gene expression analysis indicates that EBNA3 proteins have a positive effect on *RUNX3* expression [7,40]. Whether these EBNA3 proteins also increase *RUNX3* enhancer-promoter interactions however, has not been tested.

The influence of EBNA3A, 3B and 3C binding on enhancer-promoter interactions at sites shared by EBNA2 has also been examined at other gene loci that include a distal intronic site at *CTBP2* bound by all four proteins [36]. CtBP1 and 2 are transcriptional repressors that bind EBNA3A and EBNA3C and mediate the repression of a number of key cell genes including

the cyclin-dependent kinase inhibitor (CDKI) $p16^{INK4a}$ [22,23,62]. The repression of $p16^{INK4a}$ by EBNA3A and EBNA3C is a critical event required for the outgrowth of EBV-infected cells [63], so regulation of CtBP2 expression by EBV TFs may influence gene repression by EBV or modulate other functions of CtBP2. Perhaps surprisingly, EBNA3A and EBNA3B repress CtBP2 transcription (EBNA3C effects were not examined), although this is consistent with the frequent silencing of CtBP2 in B cells [36]. Repression of *CTBP2* by EBNA3A was shown to involve the disruption of interactions between the gene promoter and the EBNA3-bound distal element [36] (Figure 1). Since EBNA2 binds the *CTBP2* site, it may direct enhancer-promoter looping in the absence of EBNA3A, but this was not directly tested. ChIP re-ChIP experiments did however show that EBNA2 and EBNA3 proteins do not bind this site simultaneously, indicative of competitive binding that may influence the extent of enhancer-promoter looping [36].

Additional studies of shared binding sites examined a cluster of enhancer sites up to 44 kb downstream of the TSS of the gene encoding the cell-cycle kinase wee1 [36]. Wee1 phosphorylates and inactivates the mitotic kinase CDK1 to prevent unscheduled or inappropriate entry into mitosis. EBV deregulation of the cell-cycle through its effects on multiple CDKIs and other cell-cycle regulators plays a critical role in EBV transformation, so Wee1 regulation may also contribute to cell-cycle disruption in infected cells [64]. Specific binding of EBNA3C to *WEE1* enhancers repressed *WEE1* expression consistent with the promotion of the transition from G2 into mitosis by EBV [36,65,66]. Surprisingly, repression by EBNA3C did not disrupt enhancer-promoter interactions, but promoted their formation [36] (Figure 1). Presumably this looping brings in co-repressors that associate with EBNA3C to the gene TSS, although this has not been investigated. EBNA2 also bound *WEE1* enhancers and loss of EBNA2 binding at these *WEE1* enhancers further reduced *WEE1* expression [36]. In

198 the absence of EBNA2 it is possible that more EBNA3C is able to bind and increase the level
199 of gene repression. Re-ChIP again confirmed mutually exclusive EBNA2 and EBNA3C
200 binding at *WEE1* [36].

201

202 Competition between EBNA3 proteins and EBNA2 binding was also observed at a 6 kb
203 intergenic enhancer in the *CXCL9*, *CXCL10* and *CXCL11* chemokine gene cluster [11].
204 EBNA3A binding to this site is associated with RBPJ-dependent repression of *CXCL9* and
205 *CXCL10* transcription, PRC recruitment and H3K27me3 deposition across the locus [11].
206 Downregulation of these chemokines is likely to involve modulation of looping between this
207 enhancer and the gene promoters, but this was not tested. Interestingly, *CXCL10* can also be
208 repressed by EBNA3C along with the adjacent *CXCL11* (if not already silenced). EBNA3C
209 binding at the *CXCL9/10* enhancer was not detected, so the mechanism of this repression
210 remains unclear [29]. Downregulation of *CXCL9*, *10* and *11* may constitute an immune
211 avoidance mechanism for EBV-infected B cells *in vivo* since these chemokines function as key
212 attractors of T and NK cells. EBNA2 binding to the *CXCL9/10* enhancer reduced EBNA3A
213 binding and repression of *CXCL9* and *CXCL10* consistent with the competitive binding and
214 opposing gene regulation observed at *WEE1* [11,36].

215

216 Analysis of enhancer-promoter interactions in the repression of genes targeted by EBNA3
217 proteins alone has thus far been examined at two gene loci; the a disintegrin and
218 metalloprotease (ADAM) gene cluster containing *ADAMDEC1* and *ADAM28* and the
219 *BCL2L11* gene locus encoding the pro-apoptotic protein Bim (Figure 1). *ADAM28* and
220 *ADAMDEC1* regulate B cell adhesion and migration, so their repression may provide a survival
221 advantage to EBV-infected B cells *in vivo*. *BCL2L11* repression by EBNA3A and EBNA3C is

known to involve PRC recruitment, increased levels of H3K27me3 and lead to long-lasting epigenetic silencing through DNA methylation at *BCL2L11* promoter CpG islands [27,67]. *BCL2L11* repression is a common event in lymphoma development, often providing protection from *MYC*-driven apoptosis in lymphomas where *MYC* is overexpressed e.g. BL [68]. EBV activation of *MYC* and repression of *BCL2L11* therefore represent lymphoma driving events.

EBNA3A and EBNA3C repress *ADAMDEC1* and *ADAM28* by binding to an intergenic enhancer bound by RBPJ [29,36,69]. As observed at *WEE1* for EBNA3C, binding of EBNA3A and EBNA3C to the *ADAM* enhancer mediated gene repression via looping with the gene TSSs, presumably through the formation of a repressive hub which leads to H3K27me3 deposition across the locus [36]. EBNA3A and EBNA3C silencing of *BCL2L11* was originally thought to be mediated through binding to the *BCL2L11* promoter [28,36], but subsequent studies identified a large chromatin hub comprising multiple enhancers bound by EBNA3A and/or EBNA3C [51]. Repression of *BCL2L11* by EBNA3A and EBNA3C involves recruitment of EZH2 (PRC2) across the enhancer-promoter hub and the disruption of all enhancer-promoter interactions (Figure 1). *BCL2L11* can be de-repressed and apoptosis induced by an EZH2 inhibitor [51]. Taken together, these studies demonstrate that repression of EBNA3A and EBNA3C target genes can operate through two different mechanisms, irrespective of whether EBNA2 targets the same sites; loop promotion or loop disruption (Figure 1).

Studies on the oncogenic miR-221 and miR-222 microRNA cluster provided the first evidence that EBNA3A and EBNA3C can activate transcription by promoting enhancer-promoter looping [70]. EBNA3A and EBNA3C activate expression of these microRNAs by binding to sites in and around the transcription unit of the pri-miR from which they are processed and

promoting enhancer looping with the TSS [70] (Figure 1). Importantly induction of these miRs led to reduced expression of their key target gene $p57^{KIP2}$, a key cyclin-dependent kinase inhibitor, providing another example of how EBV regulates gene expression to perturb cell-cycle regulation in infected cells.

Conclusion

It is clear that the targeting of long-range regulatory elements by EBV TFs in B cells induces significant changes to three-dimensional enhancer-promoter interactions to modulate the expression of multiple genes involved in a variety of B cell processes. This chromatin reorganisation is likely to be critical for the transformation of B cells and can lead to cancer-driving events that include the long-term silencing of tumour suppressor genes like *BCL2L11* and may include the promotion of *MYC* gene translocations. Studying EBV enhancers has led to the identification of new regulatory hubs that may be affected in other cancers and has identified therapeutic opportunities for super-enhancer targeting or EZH2 inhibition. RBPJ plays a critical role in mediating the binding of EBV TFs to many cell gene enhancers but there is much more to learn about the roles played by other targeting co-factors and it is still unclear what determines whether a specific gene target is repressed via ‘anti-looping’ or looping.

Acknowledgements

This work was supported by Bloodwise [grant numbers 12035, 13032, 15024]; the Medical Research Council [MR/K01952X/1].

Figure caption

Figure 1. The effects of EBV transcription factors on enhancer-promoter interactions at different gene loci in B cells. The main EBV nuclear antigens (EBNAs) involved in regulation of specific genes are indicated next to the arrows. At transcribed genes the C-terminal domain of RNA polymerase II (pol II) is shown in its hyperphosphorylated state with phosphates represented as yellow spheres. EBNA 3B (3B), EBNA3A (3A) and EBNA2 (2, shown as a dimer) are shown bound to gene regulatory elements via cellular cofactors (coloured spheres). RBPJ (J) is shown at genes where it has been demonstrated to be involved in EBNA binding and gene regulation. At *MYC*, SWI/SNF complexes (S) are shown bound at upstream EBNA2 enhancers. An extruded loop is also formed upstream of *MYC* in the presence of EBNA2 as a result of interaction between two CTCF (C) bound sites. Additional coloured spheres represent various cellular co-activators or co-repressors involved in mediating enhancer-promoter interactions and gene regulation.

References

1. Miyashita EM, Yang B, Babcock GJ, Thorley-Lawson DA: **Identification of the site of Epstein-Barr virus persistence in vivo as a resting B cell**. *J Virol* 1997, **71**:4882-4891.
2. Murray RJ, Kurilla MG, Brooks JM, Thomas WA, Rowe M, Kieff E, Rickinson AB: **Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies**. *J Exp Med* 1992, **176**:157-168.
3. Cohen JI, Wang F, Mannick J, Kieff E: **Epstein-Barr virus nuclear protein 2 is a key determinant of lymphocyte transformation**. *Proc Natl Acad Sci U S A* 1989, **86**:9558-9562.
4. Tomkinson B, Robertson E, Kieff E: **Epstein-Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation**. *J Virol* 1993, **67**:2014-2025.
5. Kaye KM, Izumi KM, Kieff E: **Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation**. *Proc Natl Acad Sci U S A* 1993, **90**:9150-9154.
6. Lee MA, Diamond ME, Yates JL: **Genetic evidence that EBNA-1 is needed for efficient, stable latent infection by Epstein-Barr virus**. *J Virol* 1999, **73**:2974-2982.
7. White RE, Groves IJ, Turro E, Yee J, Kremmer E, Allday MJ: **Extensive co-operation between the Epstein-Barr virus EBNA3 proteins in the manipulation of host gene expression and epigenetic chromatin modification**. *PLoS One* 2010, **5**:e13979.
8. Robertson ES, Lin J, Kieff E: **The amino-terminal domains of Epstein-Barr virus nuclear proteins 3A, 3B, and 3C interact with RBPJ(kappa)**. *J Virol* 1996, **70**:3068-3074.
9. Zimber-Strobl U, Strobl LJ, Meitinger C, Hinrichs R, Sakai T, Furukawa T, Honjo T, Bornkamm GW: **Epstein-Barr virus nuclear antigen 2 exerts its transactivating function through interaction with recombination signal binding protein RBP-J kappa, the homologue of Drosophila Suppressor of Hairless**. *Embo J* 1994, **13**:4973-4982.
10. Johannsen E, Miller CL, Grossman SR, Kieff E: **EBNA-2 and EBNA-3C extensively and mutually exclusively associate with RBPJkappa in Epstein-Barr virus-transformed B lymphocytes**. *J Virol* 1996, **70**:4179-4183.
11. Harth-Hertle ML, Scholz BA, Erhard F, Glaser LV, Dolken L, Zimmer R, Kempkes B: **Inactivation of intergenic enhancers by EBNA3A initiates and maintains polycomb signatures across a chromatin domain encoding CXCL10 and CXCL9**. *PLoS Pathog* 2013, **9**:e1003638.
12. *Wang A, Welch R, Zhao B, Ta T, Keles S, Johannsen E: **EBNA3 proteins regulate EBNA2 binding to distinct RBPJ genomic sites**. *J Virol* 2015:DOI: 10.1128/JVI.02737-02715. The first demonstration that IRF4 is required for the binding of EBNA3C to specific target gene sites in B cells.
13. Lee S, Sakakibara S, Maruo S, Zhao B, Calderwood MA, Holthaus AM, Lai CY, Takada K, Kieff E, Johannsen E: **Epstein-Barr virus nuclear protein 3C domains necessary for lymphoblastoid cell growth: interaction with RBP-Jkappa regulates TCL1**. *J Virol* 2009, **83**:12368-12377.
14. Maruo S, Johannsen E, Illanes D, Cooper A, Zhao B, Kieff E: **Epstein-Barr virus nuclear protein 3A domains essential for growth of lymphoblasts: transcriptional regulation through RBP-Jkappa/CBF1 is critical**. *J Virol* 2005, **79**:10171-10179.

15. Maruo S, Wu Y, Ito T, Kanda T, Kieff ED, Takada K: **Epstein-Barr virus nuclear protein EBNA3C residues critical for maintaining lymphoblastoid cell growth**. *Proc Natl Acad Sci U S A* 2009, **106**:4419-4424.
16. Yalamanchili R, Tong X, Grossman S, Johannsen E, Mosialos G, Kieff E: **Genetic and biochemical evidence that EBNA 2 interaction with a 63-kDa cellular GTG-binding protein is essential for B lymphocyte growth transformation by EBV**. *Virology* 1994, **204**:634-641.
17. Zhao B, Sample CE: **Epstein-barr virus nuclear antigen 3C activates the latent membrane protein 1 promoter in the presence of Epstein-Barr virus nuclear antigen 2 through sequences encompassing an spi-1/Spi-B binding site**. *J Virol* 2000, **74**:5151-5160.
18. Johannsen E, Koh E, Mosialos G, Tong X, Kieff E, Grossman SR: **Epstein-Barr virus nuclear protein 2 transactivation of the latent membrane protein 1 promoter is mediated by J kappa and PU.1**. *J Virol* 1995, **69**:253-262.
19. Banerjee S, Lu J, Cai Q, Saha A, Jha HC, Dzeng RK, Robertson ES: **The EBV Latent Antigen 3C Inhibits Apoptosis through Targeted Regulation of Interferon Regulatory Factors 4 and 8**. *PLoS Pathog* 2013, **9**:e1003314.
20. *Paschos K, Bazot Q, Ho G, Parker GA, Lees J, Barton G, Allday MJ: **Core binding factor (CBF) is required for Epstein-Barr virus EBNA3 proteins to regulate target gene expression**. *Nucleic Acids Res* 2016.
The first report to demonstrate an interaction between EBNA3 proteins and the RUNX heterodimeric partner CBFβ. This study also demonstrates a requirement for CBFβ for EBNA3B and EBNA3C binding to specific gene elements, supporting the enrichment for RUNX motifs at EBNA3-bound human genome sites.
21. Palermo RD, Webb HM, Gunnell A, West MJ: **Regulation of transcription by the Epstein-Barr virus nuclear antigen EBNA 2**. *Biochem Soc Trans* 2008, **36**:625-628.
22. Hickabottom M, Parker GA, Freemont P, Crook T, Allday MJ: **Two nonconsensus sites in the Epstein-Barr virus oncoprotein EBNA3A cooperate to bind the co-repressor carboxyl-terminal-binding protein (CtBP)**. *J Biol Chem* 2002, **277**:47197-47204.
23. Touitou R, Hickabottom M, Parker G, Crook T, Allday MJ: **Physical and functional interactions between the corepressor CtBP and the Epstein-Barr virus nuclear antigen EBNA3C**. *J Virol* 2001, **75**:7749-7755.
24. Bazot Q, Deschamps T, Tafforeau L, Siouda M, Leblanc P, Harth-Hertle ML, Rabourdin-Combe C, Lotteau V, Kempkes B, Tommasino M, et al.: **Epstein-Barr virus nuclear antigen 3A protein regulates CDKN2B transcription via interaction with MIZ-1**. *Nucleic Acids Res* 2014, **42**:9700-9716.
25. Radkov SA, Touitou R, Brehm A, Rowe M, West M, Kouzarides T, Allday MJ: **Epstein-Barr virus nuclear antigen 3C interacts with histone deacetylase to repress transcription**. *J Virol* 1999, **73**:5688-5697.
26. Knight JS, Lan K, Subramanian C, Robertson ES: **Epstein-Barr virus nuclear antigen 3C recruits histone deacetylase activity and associates with the corepressors mSin3A and NCoR in human B-cell lines**. *J Virol* 2003, **77**:4261-4272.
27. Paschos K, Smith P, Anderton E, Middeldorp JM, White RE, Allday MJ: **Epstein-barr virus latency in B cells leads to epigenetic repression and CpG methylation of the tumour suppressor gene Bim**. *PLoS Pathog* 2009, **5**:e1000492.
28. Paschos K, Parker GA, Watanatanasup E, White RE, Allday MJ: **BIM promoter directly targeted by EBNA3C in polycomb-mediated repression by EBV**. *Nucleic Acids Res* 2012, **40**:7233-7246.

29. McClellan MJ, Khasnis S, Wood CD, Palermo RD, Schlick SN, Kanhere AS, Jenner RG, West MJ: **Downregulation of Integrin Receptor-Signaling Genes by Epstein-Barr Virus EBNA 3C via Promoter-Proximal and -Distal Binding Elements**. *J Virol* 2012, **86**:5165-5178.
30. Cotter MA, 2nd, Robertson ES: **Modulation of histone acetyltransferase activity through interaction of epstein-barr nuclear antigen 3C with prothymosin alpha**. *Mol Cell Biol* 2000, **20**:5722-5735.
31. Zimber-Strobl U, Kremmer E, Grasser F, Marschall G, Laux G, Bornkamm GW: **The Epstein-Barr virus nuclear antigen 2 interacts with an EBNA2 responsive cis-element of the terminal protein 1 gene promoter**. *Embo J* 1993, **12**:167-175.
32. Wang F, Tsang SF, Kurilla MG, Cohen JI, Kieff E: **Epstein-Barr virus nuclear antigen 2 transactivates latent membrane protein LMP1**. *J Virol* 1990, **64**:3407-3416.
33. Sung NS, Kenney S, Gutsch D, Pagano JS: **EBNA-2 transactivates a lymphoid-specific enhancer in the BamHI C promoter of Epstein-Barr virus**. *J Virol* 1991, **65**:2164-2169.
34. Le Roux A, Kerdiles B, Walls D, Dedieu JF, Perricaudet M: **The Epstein-Barr virus determined nuclear antigens EBNA-3A, -3B, and -3C repress EBNA-2-mediated transactivation of the viral terminal protein 1 gene promoter**. *Virology* 1994, **205**:596-602.
35. Zhao B, Zou J, Wang H, Johannsen E, Peng CW, Quackenbush J, Mar JC, Morton CC, Freedman ML, Blacklow SC, et al.: **Epstein-Barr virus exploits intrinsic B-lymphocyte transcription programs to achieve immortal cell growth**. *Proc Natl Acad Sci U S A* 2011, **108**:14902-14907.
36. McClellan MJ, Wood CD, Ojeniyi O, Cooper TJ, Kanhere A, Arvey A, Webb HM, Palermo RD, Harth-Hertle ML, Kempkes B, et al.: **Modulation of enhancer looping and differential gene targeting by Epstein-Barr virus transcription factors directs cellular reprogramming**. *PLoS Pathog* 2013, **9**:e1003636.
37. Schmidt SC, Jiang S, Zhou H, Willox B, Holthaus AM, Kharchenko PV, Johannsen EC, Kieff E, Zhao B: **Epstein-Barr virus nuclear antigen 3A partially coincides with EBNA3C genome-wide and is tethered to DNA through BATF complexes**. *Proc Natl Acad Sci U S A* 2015, **112**:554-559.
38. Jiang S, Willox B, Zhou H, Holthaus AM, Wang A, Shi TT, Maruo S, Kharchenko PV, Johannsen EC, Kieff E, et al.: **Epstein-Barr Virus Nuclear Antigen 3C binds to BATF/IRF4 or SPI1/IRF4 composite sites and recruits Sin3A to repress CDKN2A**. *Proc Natl Acad Sci U S A* 2014, **111**:421-426.
39. Zhou H, Schmidt SC, Jiang S, Willox B, Bernhardt K, Liang J, Johannsen EC, Kharchenko P, Gewurz BE, Kieff E, et al.: **Epstein-Barr Virus Oncoprotein Super-enhancers Control B Cell Growth**. *Cell Host Microbe* 2015, **17**:205-216.
40. *Gunnell A, Webb HM, Wood CD, McClellan MJ, Wichaidit B, Kempkes B, Jenner RG, Osborne C, Farrell PJ, West MJ: **RUNX super-enhancer control through the Notch pathway by Epstein-Barr virus transcription factors regulates B cell growth**. *Nucleic Acids Res* 2016, **44**:4636-4650.
Demonstrates that EBNA2 activates RUNX3 transcription via an upstream RBPJ-dependent super-enhancer.
41. Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA: **Master transcription factors and mediator establish super-enhancers at key cell identity genes**. *Cell* 2013, **153**:307-319.
42. Chapuy B, McKeown MR, Lin CY, Monti S, Roemer MG, Qi J, Rahl PB, Sun HH, Yeda KT, Doench JG, et al.: **Discovery and characterization of super-enhancer-**

associated dependencies in diffuse large B cell lymphoma. *Cancer Cell* 2013, **24**:777-790.
43. Wang Y, Zhang T, Kwiatkowski N, Abraham BJ, Lee TI, Xie S, Yuzugullu H, Von T, Li H, Lin Z, et al.: **CDK7-dependent transcriptional addiction in triple-negative breast cancer**. *Cell* 2015, **163**:174-186.
44. Kwiatkowski N, Zhang T, Rahl PB, Abraham BJ, Reddy J, Ficarro SB, Dastur A, Amzallag A, Ramaswamy S, Tesar B, et al.: **Targeting transcription regulation in cancer with a covalent CDK7 inhibitor**. *Nature* 2014, **511**:616-620.
45. Christensen CL, Kwiatkowski N, Abraham BJ, Carretero J, Al-Shahrour F, Zhang T, Chipumuro E, Herter-Sprie GS, Akbay EA, Altabef A, et al.: **Targeting transcriptional addictions in small cell lung cancer with a covalent CDK7 inhibitor**. *Cancer Cell* 2014, **26**:909-922.
46. Chipumuro E, Marco E, Christensen CL, Kwiatkowski N, Zhang T, Hatheway CM, Abraham BJ, Sharma B, Yeung C, Altabef A, et al.: **CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer**. *Cell* 2014, **159**:1126-1139.
47. Li W, Notani D, Rosenfeld MG: **Enhancers as non-coding RNA transcription units: recent insights and future perspectives**. *Nat Rev Genet* 2016, **17**:207-223.
48. *Liang J, Zhou H, Gerdt C, Tan M, Colson T, Kaye KM, Kieff E, Zhao B: **Epstein-Barr virus super-enhancer eRNAs are essential for MYC oncogene expression and lymphoblast proliferation**. *Proc Natl Acad Sci U S A* 2016, **113**:14121-14126. *Proc Natl Acad Sci U S A* 2016, **113**:14121-14126.
Identifies enhancer RNAs at EBV super-enhancers.
49. Hertle ML, Popp C, Petermann S, Maier S, Kremmer E, Lang R, Mages J, Kempkes B: **Differential gene expression patterns of EBV infected EBNA-3A positive and negative human B lymphocytes**. *PLoS Pathog* 2009, **5**:e1000506.
50. Kaiser C, Laux G, Eick D, Jochner N, Bornkamm GW, Kempkes B: **The proto-oncogene c-myc is a direct target gene of Epstein-Barr virus nuclear antigen 2**. *J Virol* 1999, **73**:4481-4484.
51. **Wood CD, Veenstra H, Khasnis S, Gunnell A, Webb HM, Shannon-Lowe C, Andrews S, Osborne CS, West MJ: **MYC activation and BCL2L11 silencing by a tumour virus through the large-scale reconfiguration of enhancer-promoter hubs**. *Elife* 2016, **5**.
Demonstrates EBV-specific three-dimensional reorganisation of the *MYC* locus and implicates this in the genesis of *MYC* breakpoints. Identifies a novel *BCL2L11* enhancer hub and shows that *BCL2L11* is repressed by EBV through disruption of this hub.
52. Wu DY, Krumm A, Schubach WH: **Promoter-specific targeting of human SWI-SNF complex by Epstein-Barr virus nuclear protein 2**. *J Virol* 2000, **74**:8893-8903.
53. Wu DY, Kalpana GV, Goff SP, Schubach WH: **Epstein-Barr virus nuclear protein 2 (EBNA2) binds to a component of the human SNF-SWI complex, hSNF5/Ini1**. *J Virol* 1996, **70**:6020-6028.
54. **Alver BH, Kim KH, Lu P, Wang X, Manchester HE, Wang W, Haswell JR, Park PJ, Roberts CW: **The SWI/SNF chromatin remodelling complex is required for maintenance of lineage specific enhancers**. *Nat Commun* 2017, **8**:14648.
Demonstrates a widespread role for SWI/SNF in maintaining the function of key enhancers.
55. Shi J, Whyte WA, Zepeda-Mendoza CJ, Milazzo JP, Shen C, Roe JS, Minder JL, Mercan F, Wang E, Eckersley-Maslin MA, et al.: **Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation**. *Genes Dev* 2013, **27**:2648-2662.

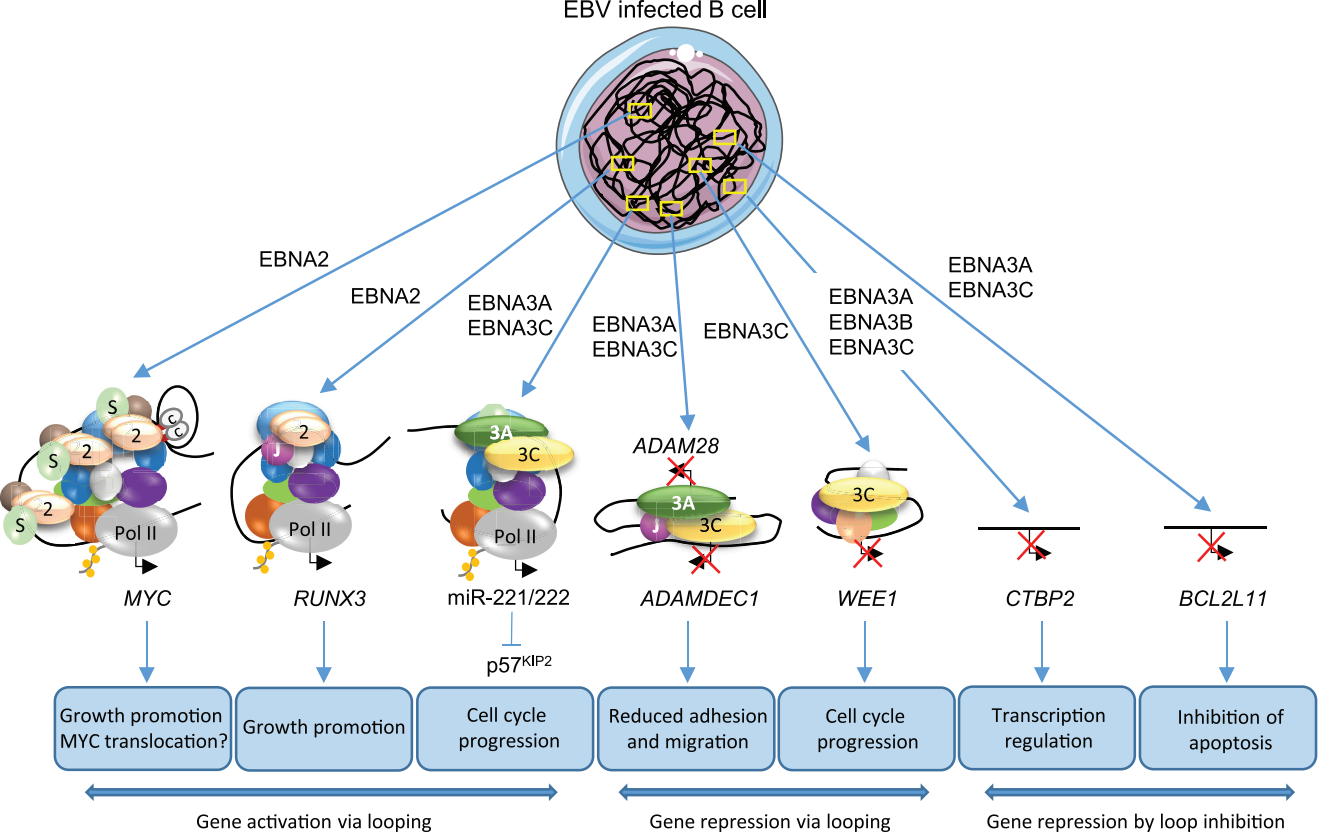
56. **Kalchschmidt JS, Bashford-Rogers R, Paschos K, Gillman AC, Styles CT, Kellam P, Allday MJ: **Epstein-Barr virus nuclear protein EBNA3C directly induces expression of AID and somatic mutations in B cells**. *J Exp Med* 2016.
Identifies the mechanism of AID induction by EBV which could contribute to the genesis of EBV associated lymphomas.
57. Wang Q, Oliveira T, Jankovic M, Silva IT, Hakim O, Yao K, Gazumyan A, Mayer CT, Pavri R, Casellas R, et al.: **Epigenetic targeting of activation-induced cytidine deaminase**. *Proc Natl Acad Sci U S A* 2014, **111**:18667-18672.
58. West MJ, Farrell PJ: **Roles of RUNX in B Cell Immortalisation**. *Adv Exp Med Biol* 2017, **962**:283-298.
59. Brady G, Whiteman HJ, Spender LC, Farrell PJ: **Downregulation of RUNX1 by RUNX3 requires the RUNX3 VWRPY sequence and is essential for Epstein-Barr virus-driven B-cell proliferation**. *J Virol* 2009, **83**:6909-6916.
60. Spender LC, Cornish GH, Sullivan A, Farrell PJ: **Expression of transcription factor AML-2 (RUNX3, CBF(alpha)-3) is induced by Epstein-Barr virus EBNA-2 and correlates with the B-cell activation phenotype**. *J Virol* 2002, **76**:4919-4927.
61. **Mifsud B, Tavares-Cadete F, Young AN, Sugar R, Schoenfelder S, Ferreira L, Wingett SW, Andrews S, Grey W, Ewels PA, et al.: **Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C**. *Nat Genet* 2015, **47**:598-606.
The development of a new genome-wide technique to specifically examine promoter interactions.
62. Skalska L, White RE, Franz M, Ruhmann M, Allday MJ: **Epigenetic repression of p16(INK4A) by latent Epstein-Barr virus requires the interaction of EBNA3A and EBNA3C with CtBP**. *PLoS Pathog* 2010, **6**:e1000951.
63. Skalska L, White RE, Parker GA, Sinclair AJ, Paschos K, Allday MJ: **Induction of p16(INK4a) Is the Major Barrier to Proliferation when Epstein-Barr Virus (EBV) Transforms Primary B Cells into Lymphoblastoid Cell Lines**. *PLoS Pathog* 2013, **9**:e1003187.
64. Allday MJ, Bazot Q, White RE: **The EBNA3 Family: Two Oncoproteins and a Tumour Suppressor that Are Central to the Biology of EBV in B Cells**. *Curr Top Microbiol Immunol* 2015, **391**:61-117.
65. Krauer KG, Burgess A, Buck M, Flanagan J, Sculley TB, Gabrielli B: **The EBNA-3 gene family proteins disrupt the G2/M checkpoint**. *Oncogene* 2004, **23**:1342-1353.
66. O'Nions J, Allday MJ: **Deregulation of the cell cycle by the Epstein-Barr virus**. *Adv Cancer Res* 2004, **92**:119-186.
67. Anderton E, Yee J, Smith P, Crook T, White RE, Allday MJ: **Two Epstein-Barr virus (EBV) oncoproteins cooperate to repress expression of the proapoptotic tumour-suppressor Bim: clues to the pathogenesis of Burkitt's lymphoma**. *Oncogene* 2008, **27**:421-433.
68. Thorley-Lawson DA, Allday MJ: **The curious case of the tumour virus: 50 years of Burkitt's lymphoma**. *Nat Rev Microbiol* 2008, **6**:913-924.
69. *Kalchschmidt JS, Gillman AC, Paschos K, Bazot Q, Kempkes B, Allday MJ: **EBNA3C Directs Recruitment of RBPJ (CBF1) to Chromatin during the Process of Gene Repression in EBV Infected B Cells**. *PLoS Pathog* 2016, **12**:e1005383.
Demonstrates that EBNA3C actually recruits RBPJ to specific sites and does not just alter RBPJ associations with histone modifying enzymes.
70. **Bazot Q, Paschos K, Skalska L, Kalchschmidt JS, Parker GA, Allday MJ: **Epstein-Barr Virus Proteins EBNA3A and EBNA3C Together Induce Expression of the Oncogenic MicroRNA Cluster miR-221/miR-222 and Ablate Expression of Its Target p57KIP2**. *PLoS Pathog* 2015, **11**:e1005031.

529 The first study to describe the mechanism of gene activation by EBNA3 proteins and to
530 identify another CDKI as an indirect target repressed by EBV.

531

532

533


EBV infected B cell
EBNA2
EBNA2
EBNA3A
EBNA3C
EBNA3A
EBNA3C
EBNA3C
EBNA3A
EBNA3B
EBNA3C
EBNA3A
EBNA3C
S
2
C
Pol II
3A
3C
J
ADAM28
MYC
RUNX3
miR-221/222
p57KIP2
ADAMDEC1
WEE1
CTBP2
BCL2L11
Growth promotion
MYC translocation?
Growth promotion
Cell cycle
progression
Reduced adhesion
and migration
Cell cycle
progression
Transcription
regulation
Inhibition of
apoptosis
Gene activation via looping
Gene repression via looping
Gene repression by loop inhibition